

Sexual selection for symmetrical male medflies (Diptera: Tephritidae) confirmed in the field

M. K. HUNT¹, C. J. NICHOLLS¹, R. J. WOOD^{1*}, A. P. RENDON² and A. S. GILBURN³

¹*School of Biological Sciences, University of Manchester, 2.205 Stopford Building, Oxford Road, Manchester M13 9PT, UK*

²*USDA-PPQ, Guatemala*

³*Department of Biology, University of Leicester, University Road, Leicester LE1 7RH, UK*

Received 20 February 2003; accepted for publication 1 August 2003

The role of fluctuating asymmetry as an indicator of fitness to females in mate choice remains controversial. Previous studies indicated that male medflies with symmetrical supra-fronto-orbital (SFO) bristles achieve relatively high mating success under laboratory conditions. Here we present data from field cage studies of wild collected medflies in Guatemala and Crete, which reveal the same association between fluctuating asymmetry in SFO bristle length and mating success as that seen in the laboratory. The experiments in Crete included males that were missing one or both of their bristles. A comparison of mating success between the three groups indicated that the mere presence of bristles did not exert a major influence. Analysis of attempted courtships suggests that the association between male mating success and FA in bristle length appears to be generated as a result of females being more likely to enter into courtships with symmetrical males, rather than through a rejection of asymmetrical males during or after it. This raises the possibility that the primary stimulus that makes a symmetrical male attractive is acting at too great a distance to depend on symmetry itself. Alternatives might include superior pheromone emissions or the occupation of a prime location within the lek. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, 81, 347–355.

ADDITIONAL KEYWORDS: *Ceratitis capitata* – fluctuating asymmetry – Mediterranean fruit fly – SFO bristles.

INTRODUCTION

Several studies have reported a negative association between fluctuating asymmetry (FA) in a sexually selected trait and male mating success (for examples see Markow & Ricker, 1992; Møller, 1992; Thornhill, 1992). Some authors have suggested that FA acts as a reliable indicator of male fitness, with symmetrical males signalling to females that they have successfully undergone development and are likely to sire offspring of a similar quality (Møller, 1990; Møller & Pomiankowski, 1993). This theory requires a heritable component to FA. A recent meta-analysis of studies investigating the heritability of FA measurements claims to have shown an overall significant heritabil-

ity (Møller & Thornhill, 1997). However, this analysis attracted an unprecedented level of criticism with several authors casting considerable doubt on the validity of these findings and the general role of FA as an indicator of fitness to females in mate decisions (Houle, 1997; Leamy, 1997; Markow & Clarke, 1997; Palmer & Strobeck, 1997; Whitlock & Fowler, 1997). In addition, others have cast doubt on the relevance of FA as an indicator of fitness to females and reported no observable effects in manipulation studies. For example, Tomkins & Simmons (1998) altered the level of FA in earwig forceps and found it had no effect on female mate choice. However, they did find that alterations of mean forceps length affected male mating success.

It appears therefore that the relationship between FA and fitness is not quite so clear-cut as was originally thought. Despite a large number of studies supporting a predictive association between FA and

*Corresponding author. E-mail: r.j.wood@man.ac.uk

fitness, there are others that have yielded equivocal or opposite findings (Owen & McBee, 1991, cited in Leung & Forbes, 1996; Eggert & Sakaluk, 1994; Goulson *et al.*, 1999; Hardesen, 2000). A recent multivariate analysis by Carchini *et al.* (2000) found that although a relationship between FA and fitness existed in the damselfly, *Ischnura elegans*, it was outweighed by the greater effect of male body size. Supporting these findings, Hunt & Simmons (1997) found no evidence that asymmetry in beetle horns signalled male quality, whilst David *et al.* (1998) found that although the size of male ornaments in stalk-eyed flies reflected condition, FA did not. It would seem that the exact relationship between FA and fitness remains to be determined.

The male Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) performs a complex courtship ritual that begins with a group of males gathering on host trees to form a lek. Males establish individual territories on the undersides of leaves (Prokopy & Hendrichs, 1979; Arita & Kaneshiro, 1985). From here they emit pheromones to attract females (Féron, 1962; Arita & Kaneshiro, 1985). At this stage the pheromone gland, or rectal epithelial sac (Arita & Kaneshiro, 1986), is erected dorsally. Females land on the upper surface of a male's leaf and must walk to the underside to approach the male. At this point the male performs a courtship ritual, involving at least five main elements (Féron, 1962; Briceño, Ramos & Eberhard, 1996). When the two flies are about 1 cm apart, the male begins to fan his wings and directs his pheromone gland under his body. After a period of fanning, the male flicks his wings backward and forward as he moves closer and rocks his head from side to side, as a prelude to jumping over the head of the female onto her abdomen, and attempting to mate with her. If the female is unreceptive at any time during courtship, including when a male has attempted a mount, she simply drops away from the leaf and flies off. This courtship sequence provides the female with olfactory, visual and acoustical stimuli (Webb *et al.*, 1983).

It has been reported that a small number of males gain the majority of matings at medfly leks (Arita & Kaneshiro, 1985; Whittier *et al.*, 1994), so there is considerable scope for sexual selection to be operating. Furthermore, medflies are sexually dimorphic for several characters that are variable in the male (Féron, 1962) and these, it is thought, could provide the female with a basis for choosing a mate.

A previous study of laboratory-reared medflies from a factory strain (i.e. one that had been mass reared during previous generations in a factory in Guatemala, producing approximately 300 million flies a week for release) revealed that male mating success under laboratory conditions was associated with the symmetry in the length of their supra-fronto-orbital

(SFO) bristles (Hunt *et al.*, 1998). These bristles are large and spatulate. Here we describe the results of two series of fieldcage studies, in Guatemala and Crete. The aim of the study in Guatemala (February 1998) was to determine if the same association between SFO bristle symmetry and mating success was found using wild flies under more natural conditions. Mating success was determined on the basis of whether or not a male was found to be copulating with a female during a 4 or 6-hour period (see Material and methods). No attempt was made to investigate the outcome of specific courtships, e.g. whether a male was rejected or not before achieving successful copulation. It was therefore unclear at what stage of male–female interaction the selection was operating, and whether by female choice via the rejection of unsuitable males, or by male competition in the form of successful males putting more effort into courtship attempts.

The aim of the study in Crete (May–July 1998) was to follow the outcome of individual courtships to determine whether FA in the length of their SFO bristles would be associated with: (1) tendency to pheromone call, (2) acceptance of courtship by visiting females, or (3) degree of male success in mating. The Crete study also included males that had lost one or both of their bristles, to determine whether extreme (i.e. absolute) asymmetry affected their likelihood of acceptance by a female. As well as considering bristle length, we also investigated the width of the spatulate end of the bristle, and the width and length of the wings.

MATERIAL AND METHODS

THE FLIES

The flies used were collected from the wild as larvae in infested fruit from coffee bushes in two farms in Retalhuleu, Guatemala, and from orange groves in Fodele, Crete. Any larvae present were allowed to develop into pupae which were transferred to cages in which the adults were allowed to eclose. The sexes were separated within 24 h of eclosion and provided with food (yeast and sugar) and water until 10 days old, to ensure that they were all sexually mature. Adults were maintained at $25 \pm 2^\circ\text{C}$, $60 \pm 10\%$ rh with a 12 : 12 h light : dark cycle in an environmentally controlled laboratory.

EXPERIMENTAL PROCEDURE

On the day prior to use, all flies were individually marked with a coloured letter printed on a small piece of paper (a method developed by D. O. McInnis in Hawaii). This was mounted on top of the thorax with a dot of acrylic paint. The flies were returned to their cages until required. Male flies (50 in Guatemala, 20 in Crete) were released into a circular net field cage

(2.5 m in height and 3 m in diameter) containing either a coffee bush (Guatemala) or an orange tree (Crete). This was done approximately 2 h after dawn.

The males were allowed 15 min to acclimatize and begin pheromone calling before an equal number of female flies were released into the cage. By this time the males had distributed themselves beneath leaves, mainly near the top of the tree, with one male to a leaf. More than 30 min usually elapsed after the females were introduced before the first of them visited a male. In Guatemala, mating pairs were collected as they formed, and the time at which mating began was noted. In Crete all individual courtships were followed from beginning to end. This required the number of males to be reduced to 20. The observations could then be made accurately by a single observer, bearing in mind the length of time during which the activity continued.

Record was kept of: (1) all females approaching each male, (2) all females engaged in courtship with each male, and (3) all copulations. In Crete, nearly every visit to a male resulted in a courtship attempt. A male was recorded as having made a courtship attempt if he managed to attract a female to his leaf and keep her standing in front of him while he initiated the sequence of wing-fanning, buzzing and head-rocking described above. The time at which each male began to pheromone call was also recorded. This was evident when the pheromone gland was extruded and elevated.

The experiments ceased after 4 or 6 h in Guatemala and Crete, respectively, by which time little, if any, courtship activity was continuing (probably due to the heat of the midday sun). All the flies were removed from the field cage and preserved in alcohol. The wet weight of the flies in Crete was recorded before preservation. The bristles and the wings of the males were

dissected and fixed onto microscope slides, and the total length and maximum width of each bristle was measured using VIDS II imaging software. Wing length was measured from the apex to the junction of the leading edge with the thorax and the width of the wing was measured at its widest point (for landmarks see Fig. 1). Only flies whose wings were intact were measured. Five replicates of each measurement were made, all by the same observer.

In Guatemala, 250 male flies were used in the trials (five replicates). Of these, 54 were not included in the analysis as they either died or were lost during the course of the experiment, mainly due to predation by ants or spiders, or they had missing bristles. Females were also predated, to approximately the same level. In Crete, 200 male flies were used (ten replicates), of which 27 died or were lost. Nearly 50% of the males used in the experiments in Crete were missing one or both of their bristles, which were lost inside the holding cages prior to the experiment. The holding cages were made of a metal mesh, in contrast to the fabric meshed cages available in Guatemala. By including these males in later analyses it was possible to investigate the effect of bristle loss on mating success.

The mean ambient temperature during the course of the experiments was 25.3°C in Guatemala (range 16–33°C) and 26.8°C in Crete (range 21–32.5°C). Mean relative humidity values were also similar: 48.9% in Guatemala and 47.5% in Crete.

STATISTICAL ANALYSIS

FA analysis was carried out according to the recommendations of Palmer & Strobeck (1986), Palmer (1994) and Swaddle, Witter & Cuthill (1994). The presence of FA was determined by establishing that the between-individual variation in the estimated

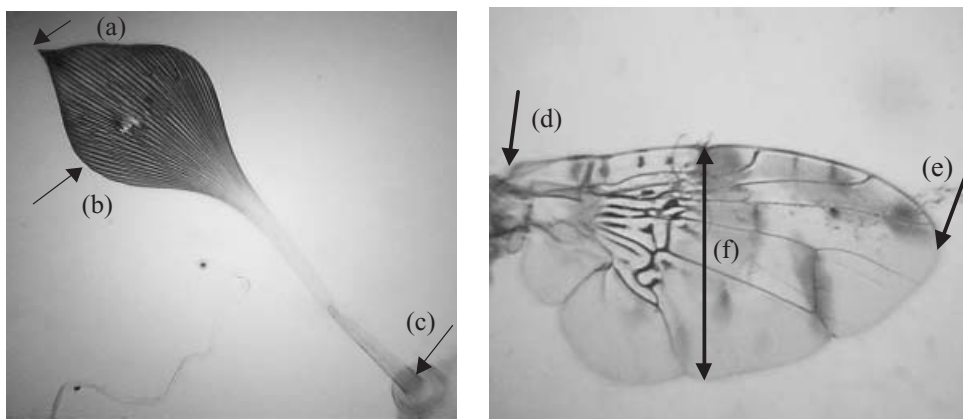


Figure 1. Dissected medfly bristle, and dissected medfly wing: (a) tip of spatulate end, (b) point of maximum width, (c) base of stalk, (d) leading edge of wing, (e) apex of wing, (f) maximum wing width.

asymmetry was significantly greater than could be accounted for by measurement error, using a mixed model ANOVA (Palmer & Strobeck, 1986; Palmer, 1994; Swaddle *et al.*, 1994). The data were tested for the presence of skew and kurtosis, and a Kolmogorov–Smirnov goodness of fit test was performed to see if the data were normally distributed, thus ruling out antisymmetry. Using *t*-tests we checked that the right minus left values [(R–L) in Palmer, 1994] had a mean not significantly different from zero, thus ruling out directional asymmetry. Even when normality tests, applied to all data routinely, suggested that the symmetry in bristle width and wing length and wing width might not be FA, the data were still used in the analysis as it is probable that forms of asymmetry other than FA may also reflect an organism's developmental stability and condition, although this idea is somewhat controversial (Graham, Freeman & Emlen, 1993; Markow, 1995; Rowe, Repasky & Palmer, 1997). Several recent studies have included in their analyses asymmetrical data showing patterns consistent with directional asymmetry or antisymmetry rather than FA (Goulson *et al.*, 1999; Hardesen, 2000).

To test whether FA was different between mated and unmated flies, *F* ratio tests were performed on the variance in the signed right minus left trait measurements in both wing and bristle dimensions (FA4 in Palmer, 1994). Associations between the level of FA and mean bristle length were tested using correlation analysis. The mean percentage of FA in the trait sizes was calculated by dividing the mean (R–L) (absolute right minus left values) by the mean trait size and multiplying by 100.

The FA data were also tested for size dependence, following the recommendations of Palmer (1994) by correlating (R–L) against either body weight or wing length for each sample of flies that mated and those that did not. If body weight had not been recorded, mean wing length was used as a measure of body size. Where a significant correlation was found, values were corrected for size dependence and tested using a scaled asymmetry index (FA6 in Palmer, 1994). This involves testing the variance in (R–L) divided by mean (R–L) between the two samples under consideration; flies that mated and those that did not. A complete description of these procedures is presented in Palmer (1994).

The acceptance rate of a male by females was calculated by dividing the number of matings by the number of courtship attempts made by that male. A courtship attempt was defined by whether the male kept the female in front of him face to face, while he initiated the ritual. Relative bristle length was calculated by dividing the mean bristle length by the mean wing length. Logistic regression analysis (SPSS, SPSS Inc., Chicago) was used to determine whether bristle

and wing lengths were associated with mating success. Standard logistic regression was used first to test for directional sexual selection and then quadratic logistic regression was used to determine if stabilizing sexual selection was operating. Significance levels were determined by the use of likelihood ratio χ^2 tests. The number of mated and unmated males possessing zero, one and two bristles was compared using a χ^2 test. The association of time with pheromone calling and acceptance rate with the number of bristles possessed by a male was determined using Kruskal–Wallis tests.

RESULTS

The between-individual variation in bristle length and width, and wing length and width, was larger than the associated measurement errors in both datasets (Table 1). In both Guatemala and Crete the signed right minus left values [(R–L) in Palmer, 1994] for bristle length were normally distributed, had means that were not significantly different from zero, and there was no significant skew or kurtosis (Table 1). However (R–L) in bristle width was not normally distributed in either dataset, and in Guatemala the mean was significantly different from zero (Table 1). There was significant positive kurtosis in (R–L) values of bristle width in both datasets, and significant positive skew in the Guatemalan flies. In the Guatemalan dataset (R–L) values for wing length were normally distributed, with a mean of zero (Table 1). (R–L) values for wing width were not normally distributed (Table 1), and there was negative skew and positive kurtosis in (R–L) values of both wing length and wing width, suggesting that the type of asymmetry being displayed by the wings was not fluctuating, but possibly directional or antisymmetrical.

GUATEMALA

In Guatemala 56% of the male flies mated successfully. Mated males were found to have longer and wider bristles and wider wings than unmated males (Table 2). However, the differences in bristle dimensions were not significant when corrections were made to allow for variation in male size (for relative bristle length $\chi^2 = 1.586$, d.f. = 2, $P = 0.208$; relative bristle width $\chi^2 = 1.489$, d.f. = 2, $P = 0.222$).

No significant correlations were observed between absolute FA, (R–L), and size in either bristle width or wing width in either of the two categories tested, mated or unmated (Table 3). However, (R–L) in bristle length was positively correlated with size in the group of males that mated (Table 3). Variance in bristle length symmetry was substantially less in mated males (Fig. 2), which showed a significantly lower FA4

Table 1. The results of a mixed model ANOVA to assess measurement error in the between-individual variation in bristle and wing dimensions; a Kolmogorov–Smirnov test to assess whether the signed right minus left values (R–L) for these dimensions were normally distributed; *t*-tests to assess whether the mean (R–L) is significantly different from zero; and the values of skew and kurtosis (significant values marked with an asterisk)

	Skew and kurtosis	Mixed model ANOVA			Kolmogorov–Smirnov				<i>t</i> -tests				
		<i>F</i>	d.f.	<i>F</i>	d.f.	<i>P</i>	<i>t</i>	<i>N</i>	<i>P</i>	skew	SE	kurtosis	SE
Bristle length	Guatemala	9.212*	195,1560	0.053	196	0.200	1.413	195	0.159	−0.271	[0.174]	0.281	[0.346]
	Crete	85.706*	97 776	0.043	95	0.200	0.716	94	0.476	−0.120	[0.247]	0.250	[0.490]
Bristle width	Guatemala	8.765*	195,1560	0.082	196	0.003	2.883	195	0.004	0.771*	[0.174]	3.464*	[0.346]
	Crete	14.939*	96 768	0.097	95	0.029	0.861	94	0.392	−0.002	[0.247]	1.942*	[0.490]
Wing length	Guatemala	14.979*	195,1560	0.061	196	0.075	0.382	195	0.703	−1.002*	[0.174]	3.991*	[0.346]
Wing width	Guatemala	3.402*	195,1560	0.108	196	<0.001	0.459	195	0.646	−0.520*	[0.174]	3.896*	[0.346]

than unmated males (Table 4) and this relationship remained after correction for size dependence (Table 4).

Mean bristle FA was 1.86% of mean bristle length compared with 2.26% in the Crete field experiment (see below). No correlation was found between the absolute asymmetries, (R–L), in bristle and wing length ($r_{196} = -0.072$, $P = 0.313$), nor between mean bristle length and (R–L) in bristle length ($F_{1,194} = 2.035$, $P = 0.155$). There was no difference in FA4 in wing length between mated and unmated males (Table 4).

CRETE

In Crete, 52% of 173 males mated. Of the 95 males with two bristles, 75 performed pheromone calling, 61 made at least one courtship attempt, and 51 mated successfully. There were no associations between bristle length and width and mating success (Table 2). There was no difference in the relative bristle length or width between males that mated and those that did not (relative bristle length, $\chi^2 = 0.027$, d.f. = 1, $P = 0.870$; relative bristle width, $\chi^2 = 0.302$, d.f. = 1, $P = 0.583$). There was also no difference in the wet weight of mated and unmated males (Table 2).

There were no significant correlations observed between absolute FA in bristle width and male weight in any of the three categories tested (Table 3). However (R–L) bristle length was positively correlated with weight in the groups of males that mated.

There was significantly less variance in bristle length FA in mated males than unmated flies (Fig. 2). Although FA4 in mated and unmated males was not quite significantly different (Table 4) ($F = 1.740$, $P = 0.056$), it became significant (in FA6) after correction for size dependence (Table 4). There was no significant difference in FA4 in bristle length between males that pheromone called during the course of the experiment and those that did not ($F = 1.034$, d.f. = 19,74, $P = 0.93$; for FA6, $F = 1.126$, $P = 0.74$). However, males with low bristle length FA4 were more likely to be visited by a female and perform a courtship ($F = 2.073$, d.f. = 33,60, $P = 0.020$). There was no association between acceptance rate and mean bristle length ($r_{61} = -0.087$, $P = 0.504$), relative bristle length ($r_{61} = -0.054$, $P = 0.679$) or male weight ($r_{61} = -0.065$, $P = 0.620$).

Overall, the number of bristles possessed by a male was not associated with its mating success ($\chi^2 = 0.94$, $P = 0.62$). However, it was associated with its capacity to retain female interest during courtship ($\chi^2 = 7.428$, $P = 0.024$, Table 5). This effect appeared to occur mainly due to the relatively low acceptance rate of males lacking both bristles.

Table 2. The mean values of wing and bristle lengths and widths in mated and unmated flies in Guatemala and Crete, and the results of logistic regressions performed on these data

		Mated mm (\pm SE)	Unmated mm (\pm SE)	χ^2	<i>P</i>
Wing length	Guatemala	3.976 (0.015)	3.934 (0.018)	2.721	0.099
	Crete	4.324 (0.016)	4.360 (0.026)	1.633	0.201
Wing width	Guatemala	2.132 (0.008)	2.096 (0.010)	7.245	0.007
	Crete	2.317 (0.011)	2.334 (0.017)	0.778	0.378
Bristle length	Guatemala	0.689 (0.003)	0.679 (0.004)	4.961	0.026
	Crete	0.758 (0.004)	0.751 (0.005)	1.170	0.279
Bristle width	Guatemala	0.219 (0.001)	0.214 (0.002)	4.786	0.029
	Crete	0.234 (0.003)	0.236 (0.005)	0.137	0.711
Weight (mg)	Crete	7.70 (0.84)	7.61 (0.83)	0.247	0.619

Table 3. Spearman's rank correlations (r_s) between male size (mean wing length in Guatemala, weight in Crete) and absolute asymmetry (R–L) in bristle and wing length and width (in mated and unmated males)

		Mated			Unmated		
Absolute asymmetry in trait		r_s	<i>N</i>	<i>P</i>	r_s	<i>N</i>	<i>P</i>
Bristle length	Guatemala	0.257	110	0.007	−0.013	86	0.908
	Crete	0.466	57	<0.001	−0.019	38	0.909
Bristle width	Guatemala	−0.103	110	0.272	−0.151	86	0.167
	Crete	0.070	57	0.607	0.079	38	0.636
Wing length	Guatemala	−0.125	110	0.193	0.208	86	0.055
Wing width	Guatemala	0.081	110	0.401	−0.020	86	0.852

Table 4. *F*-ratio tests comparing fluctuating asymmetry (FA4 or FA6) in bristle length and wing length between mated and unmated flies in Guatemala and Crete

Population	Character	FA	<i>F</i> -value	<i>P</i>
Guatemala (d.f. 85,109)	Bristle length	4	1.524	0.041
	Bristle length	6	1.595	0.024
	Wing length	4	0.732	0.128
Crete (d.f. 38,57)	Bristle length	4	1.740	0.056
	Bristle length	6	1.909	0.033

Table 5. The number mated and the acceptance rate of males with 0, 1 and 2 bristles

	0	1	2
Mated	12	27	51
Unmated	15	26	42
Acceptance rate (SE)	0.559 (0.105)	0.815 (0.052)	0.829 (0.043)

DISCUSSION

A previous study under laboratory conditions revealed that male medflies with SFO bristles symmetrical in length were more successful at mating than their asymmetrical counterparts. (Hunt *et al.*, 1998). Here we show that the same association occurs under field cage conditions at two separate locations, one in Guatemala, the second in Crete. In Guatemala we also find evidence of sexual selection in favour of long bristles, broad bristles and broad wings. In neither locality was

an association observed between SFO bristle length and bristle length FA. Hunt *et al.* (1998) found that laboratory bred males with SFO bristles of intermediate size relative to their body size had a greater mating success than males with bristles either larger or smaller relative to their body size. No such effect was observed in either series of field cage studies. The only consistent evidence of sexual selection was in respect to bristle length FA.

In Crete a large proportion of males lost one or more bristles prior to being used in the experiments. In terms of mating success, males lacking a single bristle

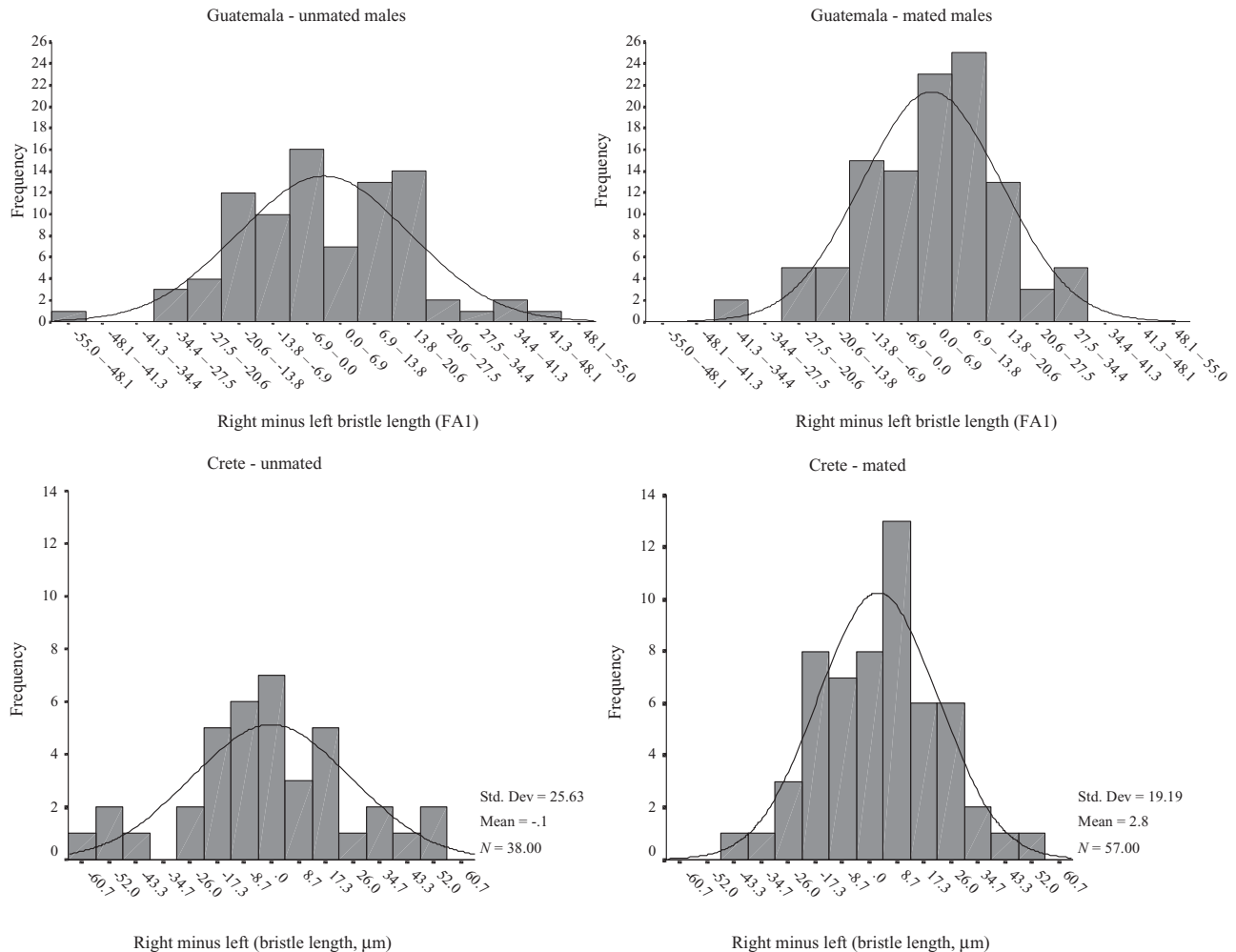


Figure 2. The variation in FA in bristle length between mated and unmated flies in Guatemala and Crete.

suffered less detriment than those lacking both, even though it made them absolutely asymmetrical. Furthermore, females were no more likely to reject the courtship attempts of males possessing only a single bristle. These data suggest that female mate choice does not operate directly on FA in bristle length, but on one or more other factors correlated with fitness. These factors need not be associated with the accidental loss of a bristle. Thus, the discovery of an association between FA and mating success should not always lead to the conclusion that females are choosing to mate with symmetrical males because of a direct appraisal of the character itself.

An indirect effect of SFO bristle length FA agrees with the findings of Mendez, Briceño & Eberhard (1998) who concluded that neither the position of the bristles on the head nor the behaviour of males during courtship supports the idea that females assess the length or the shape of the bristle directly. Females are probably unable to assess the symmetry in male bris-

tles with a necessary degree of accuracy. An important issue for the female in making such an assessment would be the degree of difference between right and left. With respect to SFO bristles, we measured this to be 1.86% of the mean bristle length in Guatemala, and 2.26% in Crete, compared with 3.69% in the earlier laboratory study (Hunt *et al.*, 1998). In this connection, Swaddle (1999) recently found that starlings were unable to discriminate asymmetrical stimuli of less than 1.8% from symmetrical patterns.

It was clear from following individual courtships in Crete of males with two bristles, that the association between bristle length FA and mating success did not occur as a result of non-random acceptance of males during courtships. Bristle length FA was, however, associated with a female being attracted towards a courting male. It was clear that the selection process in favour of symmetrical males occurred before the courtship stage, with females in Crete being more likely to alight on a leaf occupied by a symmetrical

male and observe its courtship behaviour. The question concerns the factors attracting females to visit leaves occupied by symmetrical males. Possibilities include a better quality pheromone or occupation of a prime location within the lek.

The function of SFO bristles is not known. One possibility is that they act as a species recognition signal (Hunt *et al.*, 1998). Some other species within the genus *Ceratitis* also possess such bristles, and there is considerable variation in the structure and colour of these bristles between species (White & Elson-Harris, 1992).

FA in wing length and width was studied in Guatemala but not in Crete due to extensive wing damage. No effect on mating success was found, in spite of the importance of wing movement in male courtship. Our results are in contrast to a recent study in Israel, which revealed that males with symmetrical wings were more successful (S. A. Field & B. Yuval, unpubl. data). A possible explanation for the lack of an effect of wing length symmetry in one study but not another could be connected with the fact that the type of wing measures used in these studies was slightly different. It may also be noted that in the Guatemala study, males successful in mating had significantly wider wings than unmated flies. Hence, despite differences between studies, there is some support for the conclusion that wing morphology plays a role in courtship. Its significance, and whether the effect is visual, auditory (in producing sound) or mechanical (dispersing pheromones), has yet to be determined.

We found no difference in weight between mated and unmated males. In this respect we agreed with Arita & Kaneshiro (1988) and Whittier *et al.* (1992) but disagreed with Churchill *et al.* (1986) who found that large laboratory males had higher mating success than small ones. Orozco & Lopez (1990) reported from a field study that body size was of little importance, but still found that almost half of matings in a trial of sterile, laboratory-reared males were by larger males. More recently, Blay & Yuval (1997) found that males deprived of protein, which are consequently smaller, had a lower mating success and began to mate later than protein-fed males. In a follow-up study, Yuval *et al.* (1998) looked at the effect of size, due to variation in protein feeding, on males' participation in a lek. They found no significant size difference between calling and resting males. Males of all sizes participated in the leks, although the calling males contained more proteins and sugars. The relationship between nutritional status and mating success seems an important matter for further investigation.

In our studies, both in the laboratory (Hunt *et al.*, 1998) and in the two field experiments reported here, the one consistent correlation with mating success has been bristle length FA. Even so, the relationship is not

straightforward. In this paper we have presented evidence that females do not choose their mates directly on the basis of bristle length FA. The factor that generates associations between FA and mating success has yet to be determined.

ACKNOWLEDGEMENTS

We are most grateful to Don McInnis for his advice during the Guatemalan field cage studies. We would also like to thank Dr A. Economopoulos for the use of his laboratory and facilities at the University of Crete, and Pavlos Mavrikakis for technical support. Thanks also to Hugh Jones for the use of his image analysis equipment. This work was supported by a BBSRC studentship and Socrates grant (to MKH) and a BBSRC ISIS award (to ASG).

REFERENCES

- Arita LH, Kaneshiro KY. 1985. The dynamics of the lek system and mating success in males of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). *Proceedings of the Hawaiian Entomological Society* **25**: 39–48.
- Arita LH, Kaneshiro KY. 1986. Structure and function of the rectal epithelium and anal glands during mating behavior in the Mediterranean fruit fly. *Proceedings of the Hawaiian Entomological Society* **26**: 27–30.
- Arita LH, Kaneshiro KY. 1988. Body size and differential mating success between males of two populations of the Mediterranean fruit fly (Diptera: Tephritidae). *Pacific Science* **42**: 173–177.
- Blay S, Yuval B. 1997. Nutritional correlates of reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). *Animal Behaviour* **54**: 59–66.
- Briceño RD, Ramos D, Eberhard WG. 1996. Courtship behavior of male *Ceratitis capitata* (Diptera: Tephritidae) in captivity. *Florida Entomologist* **79**: 130–143.
- Carchini G, Chiarotti F, Di Domenico M, Paganotti G. 2000. Fluctuating asymmetry, size and mating success in males of *Ischnura elegans* (Vander Linden) (Odonata: Coenagrionidae). *Animal Behaviour* **59**: 177–182.
- Churchill-Stanland C, Stanland R, Wong TY, Tanaka N, McInnis DO, Dowell RV. 1986. Size as a factor in the mating propensity of Mediterranean fruit flies, *Ceratitis capitata* (Diptera: Tephritidae), in the laboratory. *Journal of Economic Entomology* **79**: 614–619.
- David P, Hingle A, Greig D, Rutherford A, Fowler K. 1998. Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. *Proceedings of the Royal Society, London, Series B* **265**: 2211–2216.
- Eggert AK, Sakaluk SK. 1994. Fluctuating asymmetry and variation in the size of courtship food gifts in decorated crickets. *American Naturalist* **144**: 708–716.
- Féron M. 1962. L'instinct de reproduction chez la mouche Méditerranéenne des fruits. *Ceratitis capitata* Wied. (Dipt. Trypetidae). Comportement sexuel. – Comportement de

- ponte. *Revue de Pathologie Vegetale et d'Entomologie Agricole de France* **41**: 1–129.
- Goulson D, Bristow L, Elderfield E, Brinklow K, Parry-Jones B, Chapman JW.** Size, symmetry, and sexual selection in the housefly, *Musca domestica*. *Evolution* **53** (2): 527–534.
- Graham JH, Freeman DC, Emlen JM.** 1993. Antisymmetry, directional asymmetry and dynamic morphogenesis. *Genetica* **89**: 121–137.
- Hardesen S.** 2000. The role of behavioural ecology of damselflies in the use of fluctuating asymmetry as a bioindicator of water pollution. *Ecological Entomology* **25**: 45–53.
- Houle D.** 1997. Comment on 'A meta-analysis of the heritability of developmental stability' by Møller and Thornhill. *Journal of Evolutionary Biology* **10**: 17–20.
- Hunt MK, Crean CS, Wood RJ, Gilburn AS.** 1998. Fluctuating asymmetry and sexual selection in the Mediterranean fruit fly (Diptera, Tephritidae). *Biological Journal of the Linnean Society* **64**: 385–396.
- Hunt J, Simmons LW.** 1997. Patterns of fluctuating asymmetry in beetle horns: An experimental examination of the honest signalling hypothesis. *Behavioral Ecology and Sociobiology* **41** (2): 109–114.
- Leamy L.** 1997. Is development stability heritability? *Journal of Evolutionary Biology* **10**: 21–29.
- Leung B, Forbes MR.** 1996. Fluctuating asymmetry in relation to stress and fitness: effects of trait type as revealed by meta-analysis. *Ecoscience* **3**: 400–413.
- Markow TA.** 1995. Evolutionary ecology and developmental instability. *Annual Review of Entomology* **40**: 105–120.
- Markow TA, Clarke GM.** 1997. Meta-analysis of the heritability of developmental stability: a giant step backward. *Journal of Evolutionary Biology* **10**: 31–37.
- Markow TA, Ricker JP.** 1992. Male size, developmental stability, and mating success in natural populations of three *Drosophila* species. *Heredity* **69**: 122–127.
- Mendez V, Briceño RD, Eberhard WG.** 1998. Functional significance of the capitate supra-fronto-orbital bristles of male medflies (*Ceratitis capitata*) (Diptera: Tephritidae). *Journal of the Kansas Entomological Society* **71**: 164–174.
- Møller AP.** 1990. Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality. *Animal Behaviour* **40**: 1185–1187.
- Møller AP.** 1992. Female swallow preference for symmetrical male sexual ornaments. *Nature (London)* **357**: 238–240.
- Møller AP, Pomiankowski A.** 1993. Fluctuating asymmetry and sexual selection. *Genetica* **89**: 267–279.
- Møller AP, Thornhill R.** 1997. A meta-analysis of the heritability of developmental stability. *Journal of Evolutionary Biology* **10**: 1–16.
- Orozco D, Lopez RO.** 1990. Mating competitiveness of wild and laboratory mass-reared medflies: effect of male size. In: Aluja M, Liedo P, eds. *Fruit flies, biology and management*. New York: Springer-Verlag, 185–188.
- Owen RD, McBee K.** 1991. Analysis of asymmetry and morphometric variation in natural populations of chromosome-damaged mice. *Texas Journal of Science*. **42**: 319–332.
- Palmer AR.** 1994. Fluctuating asymmetry analyses: a primer. In: Markow TA, ed. *Developmental instability: its origins and evolutionary implications*. Dordrecht: Kluwer.
- Palmer AR, Strobeck C.** 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology and Systematics* **17**: 391–421.
- Palmer AR, Strobeck C.** 1997. Fluctuating asymmetry and developmental instability: heritability of observable variation versus heritability of inferred cause. *Journal of Evolutionary Biology* **10**: 39–49.
- Prokopy RJ, Hendrichs J.** 1979. Mating behavior of *Ceratitis capitata* on a field-caged host tree. *Annals of the Entomological Society of America* **72**: 642–648.
- Rowe L, Repasky RR, Palmer AR.** 1997. Size-dependent asymmetry: fluctuating asymmetry versus antisymmetry and its relevance to condition-dependent signalling. *Evolution* **51** (5): 1401–1408.
- Swaddle JP, Witter MS, Cuthill IC.** 1994. The analysis of fluctuating asymmetry. *Animal Behaviour* **48**: 986–989.
- Swaddle, JP.** 1999. Limits to length asymmetry detection in starlings: implications for biological signalling. *Proceedings of the Royal Society, London, Series B* **266**: 1299–1303.
- Thornhill R.** 1992. Female preference for the pheromone of males with low fluctuating asymmetry in the Japanese scorpionfly (*Panorpa japonica*: Mecoptera). *Behavioural Ecology* **3**: 277–283.
- Tomkins JL, Simmons LW.** 1998. Female choice and manipulations of forceps size and symmetry in the earwig *Forficula auricularia*. *Animal Behaviour* **56**: 347–356.
- Webb JC, Calkins CO, Chambers DL, Schweinbacher W, Russ K.** 1983. Acoustic aspects of behaviour of Mediterranean fruit fly, *Ceratitis capitata*: analysis and identification of courtship sounds. *Entomologia Experimentalis et Applicata* **33**: 1–8.
- White IM, Elson-Harris MM.** 1992. *Fruit flies of economic significance: their identification and bionomics*. Oxford: CAB International, in association with ACIAR.
- Whitlock MC, Fowler K.** 1997. The heritability of studies of instability. *Journal of Evolutionary Biology* **10**: 63–67.
- Whittier TS, Kaneshiro KY, Prescott LD.** 1992. Mating behavior of Mediterranean fruit flies (Diptera: Tephritidae) in a natural environment. *Annals of the Entomological Society of America* **85**: 214–218.
- Whittier TS, Nam FY, Shelly TE, Kaneshiro KY.** 1994. Male courtship success and female discrimination in the Mediterranean fruit fly (Diptera: Tephritidae). *Journal of Insect Behaviour* **7**: 159–170.
- Yuval B, Kaspi R, Shloush S, Warburg MS.** 1998. Nutritional reserves regulate male participation in Mediterranean fruit fly, leks. *Ecological Entomology* **23**: 211–215.

